Clinical Microbiology and Infection 26 (2020) 1413.e1–1413.e7

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

# Isothermal microcalorimetry minimal inhibitory concentration testing in extensively drug resistant Gram-negative bacilli: a multicentre study☆

C. Tellapragada [1], B. Hasan [1], A. Antonelli [3, 4], A. Maruri [5], C. de Vogel [6], D. Gijón [5], M. Coppi [3, 4], A. Verbon [6], W. van Wamel [6], G.M. Rossolini [3, 4], R. Cantón [5], C.G. Giske [1, 2, *]

[1] Department of Laboratory Medicine, Division of Clinical Microbiology, Karolinska Institute, Stockholm, Sweden
[2] Division of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden
[3] Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy
[4] Clinical Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italy
[5] Servicio de Microbiología. Hospital Universitario Ramón y Cajal e Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain
[6] Department of Medical Microbiology and Infectious Diseases, Erasmus MC, Rotterdam, the Netherlands

ARTICLE INFO

*Article history:*
Received 30 September 2019
Received in revised form
27 December 2019
Accepted 21 January 2020
Available online 30 January 2020

Editor: P. T. Tassios

*Keywords:*
Broth microdilution
Categorical agreement
Essential agreeme
Extensively drug-resistant (XDR) bacteria
Isothermal microcalorimetry
Minimal inhibitory concentration

ABSTRACT

*Objectives:* To evaluate the performance of an isothermal microcalorimetry (IMC) method for determining the MICs among extensively drug-resistant Gram-negative bacilli.
*Methods:* A collection of 320 clinical isolates ($n = 80$ of each) of *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* from Sweden, Spain, Italy and the Netherlands were tested. The MICs were determined using the IMC device calScreener (Symcel, Stockholm, Sweden) and ISO-broth microdilution as the reference method. Essential agreement, categorical agreement, very major errors (VME), major errors (ME) and minor (mE) errors for each antibiotic were determined.
*Results:* Data from 316 isolates were evaluated. Four errors (two ME, one VME, one mE) among 80 *K. pneumoniae,* six errors (four ME, one VME, one mE) among 79 *E. coli,* 15 errors (seven VME, three ME, five mE) among 77 *P. aeruginosa* and 18 errors (12 VME, two ME, four mE) among 80 *A. baumannii* were observed. Average essential agreement and categorical agreement of the IMC method were 96.6% (95% confidence interval, 94.2–99) and 97.1% (95% confidence interval, 95.4–98.5) respectively when the MICs were determined at the end of 18 hours. Categorical agreement of the IMC method for prediction of MIC by the end of 8 hours for colistin, meropenem, amikacin, ciprofloxacin and piperacillin/tazobactam were 95%, 91.4%, 94%, 95.2% and 93.7% respectively.
*Conclusions:* The IMC method could accurately determine the MICs among extensively drug-resistant clinical isolates of *E. coli, K. pneumoniae, P. aeruginosa* and *A. baumannii* isolates. **C. Tellapragada, Clin Microbiol Infect 2020;26:1413.e1–1413.e7**
© 2020 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

## Introduction

Early detection of microbial aetiology and initiation of pathogen-specific antimicrobial therapy are crucial pillars for successful management of clinical infections. Despite the advent of DNA-based detection methods for pathogens and antimicrobial resistance genes directly in clinical specimens, microbiologic culture followed by antimicrobial susceptibility testing (AST) remains the reference method for diagnosis of bacterial infections. For AST and determining the MICs against important antimicrobial agents, the majority of clinical laboratories worldwide use well-established methods such as the disc diffusion test and broth microdilution (BMD) assays. With the global upsurge in the prevalence of infections, particularly due to multidrug-resistant bacteria, quantifying antimicrobial resistance by determining the MICs becomes

☆ Presented in part at the 29th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), April 2019, Amsterdam, The Netherlands.

* Corresponding author. C.G. Giske, Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institute, Alfred Nobels Allé 8, 14183 Stockholm, Sweden.
*E-mail address:* christian.giske@ki.se (C.G. Giske).

https://doi.org/10.1016/j.cmi.2020.01.026
1198-743X/© 2020 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

indispensable. Currently available automated or semiautomated systems for AST and MIC determination against commonly used antimicrobial agents often remain prone to error or less calibrated compared to BMD [1,2]. Moreover, the majority of these systems provide results by the end of 16 to 18 hours of incubation, which is nearly the same amount of time required using manual methods. In this context, clinical laboratories continue to face the challenge of providing timely and reliable MIC results, which in turn are crucial for effective antimicrobial stewardship programmes.

Isothermal microcalorimetry (IMC) is a well-acknowledged method for measuring the energy released during metabolic processes in a biological system. Metabolically active bacteria generate energy which is proportional to their growth rate in a given culture system. IMC is capable of measuring the energy released in an isothermal, closed system at microwatt levels and this property makes it a potential tool to study the growth kinetics of bacteria [3,4]. IMC has several advantages compared to traditional assay technologies for studying AST. The technology has a very high sensitivity compared to optical readouts, enabling a potential early detection of inhibition. The IMC technology can be used under a variety of media conditions, and being a label-free technology, it can also be applied for measurement of samples with complex geometries such as turbid or complex samples. IMC has been used to study the mode of action of various antimicrobial agents when used at subinhibitory concentrations [5]. Exposure of bacteria to antimicrobials influences their metabolism and physiology, thereby affecting the energy released. Isothermal microcalorimetry is a real-time monitoring of the energy release/heat flow that can help us better understand the influence of an antimicrobial agent on the bacterial growth given time-resolved data. There are currently few data regarding the accuracy and speed of IMC in predicting the MICs in the published literature.

We conducted a multicentre evaluation of the performance of a 48-well IMC device calScreener (Symcel, Stockholm, Sweden), to determine the MICs among clinical isolates of *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* against clinically important antimicrobial agents.

## Materials and methods

### *Study details*

We present here the data from an ongoing (January 2018 to December 2019) multicentre laboratory-based evaluation study. Study sites were the clinical microbiologic laboratories at Karolinska University Hospital, Sweden; Azienda Universitario Ospedaliera Careggi, Italy; Hospital Universitario Ramon y Cajal, Spain; and Erasmus Medical Center, Netherlands.

### *Study isolates, antibiotics and media*

Nonreplicate clinical isolates of *E. coli* ($n = 80$), *Klebsiella pneumoniae* ($n = 80$), *Pseudomonas aeruginosa* ($n = 80$) and *A. baumannii* ($n = 80$) with pre-determined antimicrobial susceptibility profiles were included in the study. Multiple isolates from known ongoing outbreaks were avoided. Challenge isolates included in the present study from all the four study sites were chosen to overrepresent resistance. Reference strains of *E. coli* (ATCC 25922 and NCTC 13476), *K. pneumoniae* (NCTC 13438), *P. aeruginosa* (ATCC 28753) and *A. baumannii* (ATCC 19606) were used for the initial standardization and reproducibility testing of the protocol for MIC determination using calScreener. Antibiotics used in the present study with their concentrations are listed in Table 1. Antibiotic powders used in the present study were procured from Sigma-Aldrich (Sweden). Mueller-Hinton broth (MHB) (Sigma-Aldrich) was used as the standard growth medium through the study. Culture media and working solutions of antibiotics used for MIC determination were prepared according to the guidelines from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) v.5.0 (http://eucast.org/ast_of_bacteria/media_preparation/).

**Table 1**
Antibiotics and concentration range

| Microorganism | Drug (concentration range) |
|---|---|
| *Escherichia coli* and *Klebsiella pneumoniae* | Amikacin (4–16 mg/L)<br>Cefotaxime (0.12–4 mg/L)<br>Ciprofloxacin (0.03–1 mg/L)<br>Meropenem (0.06–16 mg/L)<br>Piperacillin/tazobactam[a] (2–32 mg/L) |
| *Pseudomonas aeruginosa* | Amikacin (4–16 mg/L)<br>Ceftazidime (4–16 mg/L)<br>Cefepime (4–16 mg/L)<br>Ciprofloxacin (0.25–2 mg/L)<br>Colistin (1–8 mg/L)<br>Meropenem (1–32 mg/L)<br>Piperacillin/tazobactam[a] (8–32 mg/L) |
| *Acinetobacter baumannii* | Amikacin (2–16 mg/L)<br>Ciprofloxacin (0.25–4 mg/L)<br>Colistin (0.5–8 mg/L)<br>Meropenem (0.5–16 mg/L)<br>Minocycline (0.5–8 mg/L)<br>Sulbactam (2–16 mg/L) |

[a] Fixed concentration of tazobactam at 4 mg/L was used throughout.

### *Inoculum and antibiotic preparation for calScreener*

BMD solutions were prepared in 48- or 96-well microtitre plates. For each antibiotic, a two-fold dilution series was prepared following the concentration ranges on Table 1 in a total volume of 150 μL at double the final concentration used. Bacterial suspensions were prepared in MHB and matched to 0.5 McFarland and diluted 1:100 in MHB. A total of 150 μL of bacterial suspensions was inoculated in each well containing antibiotics. In each plate there were control wells: one negative control that had only MHB, two positive control wells that had MHB and the bacterial inoculum but not the antibiotics. From this master plate, 120 μL of each sample ($5 \times 10^5$ CFU/mL bacterial suspension with or without antibiotic) were transferred to sterile plastic inserts designed to fit in titanium measurement vials (calWell; Symcel) as a part of the calScreener sample handling system [6,7]. The inserts were placed in the calWells, sealed and loaded in the calScreener. The calWells serving as thermodynamic references were loaded with 120 μL of MHB. Real-time measurement of the heat produced from each calWells was carried out at 37 ± 0.001°C in the calScreener for a minimum duration of 18 hours. The remaining samples in the microtitre plate were incubated at 37°C for 16 to 18 hours.

### *Performance evaluation of IMC method*

ISO-broth microdilution (ISO-BMD) was used as the reference method for evaluation of the IMC method. Interpretation of the MIC results was carried out using the EUCAST clinical breakpoint criteria (v.9.0, January 2019). Isolates that produced discrepant results (very major errors and major errors) using the IMC method were subjected to repeat testing (three times) by the reference method. Overall essential and categorical agreements of the IMC method were analysed according to the criteria in ISO standards 20776-1 and 20776-2 [8].

*Data analysis*

The heat produced in the calWells was continuously measured and expressed as heat flow over time (μW) using the manufacturer's calView software (Symcel). The initial 30 minutes of incubation after signal stabilization was used as baseline to correct for internal sample variation. Isolates that released heat of at least >10 μW, which allowed us to set a stable baseline, were all included in the analysis. The total heat released by each isolate was calculated for every 30-minute interval; the relative heat was then defined as the total heat ratio between samples exposed to antibiotics and the positive control (no antibiotic). When a ratio surpassed 20%, it was considered positive growth, and if it did not surpass the cutoff for the duration of the assay (8 or 18 hours), it was considered negative. MIC was defined as the antimicrobial concentration that inhibited the heat release [9]. Statistical analysis was performed by GraphPad Prism 8.0 software (GraphPad Software, La Jolla, CA, USA).

## Results

*Evaluation of the calScreener using reference strains*

MICs against the panel of antibiotics (Table 1) for each of the five reference strains were determined three times independently at all four study sites. The reference strains used in the present study covered the quality control (QC) for all drugs with published QC ranges. All of the four study sites obtained on-scale results for the reference strains for the drugs tested within the published QC ranges for those organisms. Overall, the average categorical and essential agreements between both the methods were 91.6% and 97.2% respectively. There was a high reproducibility between sites determining the MIC values: in 95.3% of the tests, all four sites determined the same MIC using the calScreener, and in 99% of all tests, the MIC was determined within a single twofold dilution difference.

*Evaluation of calScreener using clinical isolates*

We tested 320 clinical isolates comprising 80 of each *E. coli, K. pneumoniae, P. aeruginosa* and *A. baumannii* using both IMC and ISO-BMD methods. Distribution of the study isolates based on their MICs against the tested antibiotics using ISO-BMD method is depicted in Table 2. Among these 320 isolates, IMC data from 316 (98.7%) could be analyzed and compared to the ISO-BMD results. IMC data from three *P. aeruginosa* and one *E. coli* isolate could not be analyzed as a result of the very low amount of heat released (<10 μW) by these isolates. For the 316 isolates included in the analysis, average essential and categorical agreements of the IMC were 96.6% (95% confidence interval, 94.2–99) and 97.1% (95% confidence interval, 95.4–98.5) respectively. Total number of isolates tested against each antibiotic and the percentage of essential and categorical agreements observed are listed in Table 3.

We deduced the error rates of the IMC method among each of the four bacterial species included in the study. Six errors (four major errors (ME), one very major errors (VME) and one minor error (mE)) were observed among 79 *E. coli* isolates tested against five antibiotics, with an overall error rate of 1.5% (6/395). Four errors (two ME and one each of VME and mE) were observed among 80 *K. pneumoniae* isolates tested against five antibiotics, with an overall error rate of 1% (4/400). Fifteen errors (seven VME, three ME, five mE) were observed among 77 *P. aeruginosa* isolates tested against seven antibiotics, with an overall error rate of 2.7% (15/539).

**Table 2**
Distribution of study isolates based on MICs against tested antibiotics using reference method

| Antimicrobial agent and microorganism | No. of isolates with MIC (mg/L): | | | | | | | | | | | | Dilution tested (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ≤0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | >32 | |
| Piperacillin/tazobactam | | | | | | | | | | | | | |
| *Escherichia coli* | — | — | — | — | — | — | 45 (56) | 5 (6.2) | 3 (3.7) | 1 (1.2) | 4 (5) | 22 (27.5) | ≤2 to >32 |
| *Klebsiella pneumoniae* | — | — | — | — | — | — | 30 (37) | 7 (8.7) | 7 (8.7) | 2 (2.5) | 2 (2.5) | 32 (40) | ≤2 to >32 |
| *Pseudomonas aeruginosa* | — | — | — | — | — | — | — | — | 50 (62) | 6 (7.5) | 9 (11) | 15 (19) | ≤8 to >32 |
| Ciprofloxacin | | | | | | | | | | | | | |
| *E. coli* | 37 (46.2) | 1 (1.2) | 1 (1.2) | 5 (6.2) | 1 (1.2) | 0 | 35 (43.7) | — | — | — | — | — | ≤0.03 to >1 |
| *K. pneumoniae* | 34 (42) | 1 (1.2) | 5 (6.2) | 1 (1.2) | 0 | 1 (1.2) | 38 (47.5) | — | — | — | — | — | ≤0.03 to >1 |
| *P. aeruginosa* | | | | 40 (50) | 3 (3.7) | 5 (6.2) | 2 (2.5) | 30 (37) | — | — | — | — | ≤0.25 to >2 |
| *Acinetobacter baumannii* | | | | 26 (32) | 5 (6.2) | 2 (2.5) | 2 (2.5) | 1 (1.2) | 36 (45) | — | — | — | ≤0.25 to >4 |
| Amikacin | | | | | | | | | | | | | |
| *E. coli* | — | — | — | — | — | — | — | 63 (78) | 7 (8.7) | 4 (5) | 6 (7.5) | — | ≤4 to >16 |
| *K. pneumoniae* | — | — | — | — | — | — | — | 61 (76) | 6 (7.5) | 5 (6.2) | 8 (10) | — | ≤4 to >16 |
| *P. aeruginosa* | — | — | — | — | — | — | — | 51 (64) | 6 (7.5) | 6 (7.5) | 17 (21) | — | ≤4 to >16 |
| *A. baumannii* | — | — | — | — | — | — | 26 (32.5) | 15 (19) | 3 (3.7) | 4 (5) | 32 (40) | — | ≤2 to >16 |
| Meropenem | | | | | | | | | | | | | |
| *E. coli* | — | 57 (71) | 3 (3.7) | 1 (1.2) | 3 (3.7) | 2 (2.5) | 5 (6.2) | 2 (2.5) | 2 (2.5) | 3 (3.7) | 2 (2.5) | — | 0.06 to >16 |
| *K. pneumoniae* | — | 48 (60) | 2 (2.5) | 1 (1.2) | 2 (2.5) | 3 (3.7) | 3 (3.7) | 1 (1.2) | 3 (3.7) | 7 (8.7) | 10 (12.5) | — | 0.06 to >16 |
| *P. aeruginosa* | — | — | — | — | — | 46 (57) | 3 (3.7) | 3 (3.7) | 3 (3.7) | 3 (3.7) | 8 (10) | 14 (17.5) | ≤1 to >32 |
| *A. baumannii* | — | — | — | — | 33 (41) | 9 (11) | 0 | 0 | 2 (2.5) | 7 (8.7) | 29 (36) | — | ≤0.5 to >16 |
| Cefotaxime | | | | | | | | | | | | | |
| *E. coli* | — | — | 40 (50) | 2 (2.5) | 2 (2.5) | 2 (2.5) | 0 | 3 (3.7) | 31 (39) | — | — | — | ≤0.12 to >4 |
| *K. pneumoniae* | — | — | 34 (42) | 2 (2.5) | 4 (5) | 1 (1.2) | 0 | 1 (1.2) | 38 (47) | — | — | — | ≤0.12 to >4 |
| Colistin | | | | | | | | | | | | | |
| *P. aeruginosa* | — | — | — | — | — | 36 (45) | 37 (46) | 4 (5) | 0 | 3 (3.7) | — | — | ≤1 to >8 |
| *A. baumannii* | — | — | — | — | 27 (34) | 24 (30) | 16 (20) | 5 (6.2) | 2 (2.5) | 6 (7.5) | — | — | ≤0.5 to >8 |
| Minocycline | | | | | | | | | | | | | |
| *A. baumannii* | — | — | — | — | 33 (41) | 8 (10) | 9 (11) | 7 (8.7) | 8 (10) | 15 (19) | — | — | ≤0.5 to >8 |
| Sulbactam | | | | | | | | | | | | | |
| *A. baumannii* | — | — | — | — | — | — | 34 (42) | 4 (5) | 5 (6.2) | 9 (11) | 28 (35) | — | ≤2 to >16 |
| Cefepime | | | | | | | | | | | | | |
| *P. aeruginosa* | — | — | — | — | — | — | — | 42 (52.5) | 12 (15) | 5 (6.2) | 21 (26) | — | ≤4 to >16 |
| Ceftazidime | | | | | | | | | | | | | |
| *P. aeruginosa* | — | — | — | — | — | — | — | 47 (59) | 4 (5) | 8 (10) | 21 (26) | — | ≤4 to >16 |

**Table 3**
Overall performance characteristics of IMC for determination of MICs against ten antimicrobial agents

| Characteristic | COL | CAZ | FEP | MER | AMK | CIP | PTZ | CTX | SUL[a] | MIN[b] |
|---|---|---|---|---|---|---|---|---|---|---|
| No. of isolates tested | 157 | 77 | 77 | 316 | 316 | 316 | 236 | 159 | 80 | 80 |
| No. of isolates with concordant results[c] | 151 | 76 | 75 | 309 | 313 | 310 | 236 | 156 | 73 | 72 |
| Essential agreement (%) | 96.1 | 98.7 | 97.4 | 97.7 | 99 | 98.1 | 100 | 98.1 | 91.2 | 90 |
| No. of isolates that are: | | | | | | | | | | |
| Resistant | 19 | 27 | 23 | 89 | 77 | 160 | 83 | 72 | 42 | 29 |
| Susceptible | 138 | 50 | 54 | 221 | 239 | 125 | 153 | 87 | 38 | 51 |
| Susceptible IE | — | — | — | 6 | — | 31 | — | — | — | — |
| VME, *n* (%) | 4 (21) | 0 | 3 (13) | 0 | 4 (5.1) | 0 | 1 (1.2) | 0 | 3 (7.1) | 6 (20.6) |
| ME, *n* (%) | 2 (1.4) | 1 (2) | 1 (1.8) | 1 (0.4) | 2 (0.8) | 3 (2.4) | 0 | 1 (1.1) | 0 | 0 |
| mE, *n* (%) | 0 | 0 | 0 | 7 (2.2) | 0 | 4 (1.2) | 0 | 0 | 0 | 0 |
| Categorical agreement (%) | 96.1 | 98.7 | 94.8 | 97.4 | 98.1 | 97.7 | 99.5 | 99.3 | 96.2 | 92.5 |

AMK, amikacin; CAZ, ceftazidime; CIP, ciprofloxacin; COL, colistin; CTX, cotrimoxazole (sulfamethoxazole/trimethoprim); FEP, cefepime; IE, increased exposure; IMC, isothermal microcalorimetry; ME, major error; mE, minor error; MER, meropenem; MIN, minocycline; PTZ, piperacillin/tazobactam; SUL, sulbactam; VME, very major.
[a] Tentative epidemiologic cutoff 4 mg/L.
[b] Tentative epidemiologic cutoff 2 mg/L.
[c] Isolates with same and/or MICs within 1 $\log_2$ dilution compared to ISO-broth microdilution.

Eighteen errors (12 VME, two ME, four mE) were observed among the 80 *A. baumannii* isolates tested against six antibiotics, with an overall error rate of 3.7% (18/480).

In the absence of data regarding breakpoints and epidemiologic cutoffs for minocycline and sulbactam, we assessed the performance of the IMC method using two tentative breakpoints for each drug, 2 and 4 mg/L for minocycline and 4 and 8 mg/L for sulbactam. The IMC method generated erroneous results more frequently while testing *A. baumannii* isolates by producing false susceptible results against sulbactam (7.1% and 21.6% using 4 and 8 mg/L as the breakpoints respectively) and minocycline (20.6% and 31.8% using 2 and 4 mg/L as the breakpoints respectively). Performance characteristics of the IMC method among the four bacterial species against individual antibiotics tested in the present study are depicted in Table 4. For brevity, comparison of the MICs determined using both the methods against selected antibiotics are depicted in Fig. 1.

*Time to detection of MIC using the IMC method*

Time to detect the metabolic activity for each of the four bacterial species included in the study was determined. Median time for detection of a stable metabolic activity among *E. coli, K. pneumoniae, P. aeruginosa* and *A. baumannii* were 3, 3.5, 6 and 4 hours respectively (Fig. 2(a)). Overall time to detect the MIC of each antibiotic among each of the four bacterial species is depicted in Figs. 2(b–e). We further compared the categorical errors of the IMC method at two different time points (by the end of 8 and 18 hours) for five important antibiotics. Average categorical agreement between IMC method at 8 hours and the reference method for colistin, meropenem, amikacin, ciprofloxacin and piperacillin/tazobactam were 95%, 91.4%, 94%, 95.2% and 93.7% respectively. Underestimation of resistance for all the antibiotics tested was observed when the MICs were predicted at 8 hours using the IMC data (Fig. 3).

**Table 4**
Performance characteristics of IMC method among four bacteria species tested

| Organism and performance characteristics | COL | CAZ | FEP | MER | AMK | CIP | PTZ | CTX | SUL | MIN |
|---|---|---|---|---|---|---|---|---|---|---|
| *Escherichia coli* (*n* = 79) | NT | NT | NT | | | | | | NT | NT |
| Resistant isolates (*n*) | | | | 6 | 9 | 34 | 26 | 33 | | |
| EA (%) | | | | 97.4 | 100 | 100 | 100 | 100 | | |
| CA (%) | | | | 98.7 | 97.4 | 96.2 | 100 | 100 | | |
| VME, *n* (%) | | | | 0 | 1 (12.5) | 0 | 0 | 0 | | |
| ME, *n* (%) | | | | 0 | 1 (1.4) | 3 (6.6) | 0 | 0 | | |
| mE, *n* (%) | | | | 1 (1.2) | 0 | 0 | 0 | 0 | | |
| *Klebsiella pneumoniae* (*n* = 80) | NT | NT | NT | | | | | | NT | NT |
| Resistant isolates (*n*) | | | | 20 | 13 | 39 | 36 | 39 | | |
| EA (%) | | | | 96.2 | 100 | 95 | 100 | 96.2 | | |
| CA (%) | | | | 97.5 | 100 | 100 | 98.7 | 98.7 | | |
| VME, *n* (%) | | | | 0 | 0 | 0 | 1 (2.7) | 0 | | |
| ME, *n* (%) | | | | 1 (1.6) | 0 | 0 | 0 | 1 (2.4) | | |
| mE, *n* (%) | | | | 1 (1.2) | 0 | 0 | 0 | 0 | | |
| *Pseudomonas aeruginosa* (*n* = 77) | | | | | | | | NT | NT | NT |
| Resistant isolates (*n*) | 6 | 27 | 23 | 25 | 19 | 33 | 21 | | | |
| EA (%) | 98.7 | 98.7 | 97.4 | 97.4 | 98.7 | 100 | 100 | | | |
| CA (%) | 96.1 | 98.6 | 94.8 | 93.5 | 97.4 | 100 | 100 | | | |
| VME, *n* (%) | 2 (33.3) | 0 | 3 (13) | 0 | 2 (10.5) | 0 | 0 | | | |
| ME, *n* (%) | 1 (1.4) | 1 (2) | 1 (1.8) | 0 | 0 | 0 | 0 | | | |
| mE, *n* (%) | 0 | 0 | 0 | 5 (6.5) | 0 | 0 | 0 | | | |
| *Acinetobacter baumannii* (*n* = 80) | | NT | NT | | | | NT | NT | | |
| Resistant isolates (*n*) | 13 | | | 38 | 36 | 54 | | | 42 | 29 |
| EA (%) | 96.2 | | | 100 | 97.5 | 97.5 | | | 91.2 | 90 |
| CA (%) | 96.2 | | | 100 | 97.5 | 95 | | | 96.2 | 92.5 |
| VME, *n* (%) | 2 (15.3) | | | 0 | 1 (2.7) | 0 | | | 3 (7.1) | 6 (20.6) |
| ME, *n* (%) | 1 (1.4) | | | 0 | 1 (2.2) | 0 | | | 0 | 0 |
| mE, *n* (%) | 0 | | | 0 | 0 | 4 (5) | | | 0 | 0 |

AMK, amikacin; CA, categorical agreement; CAZ, ceftazidime; CIP, ciprofloxacin; COL, colistin; CTX, cotrimoxazole (sulfamethoxazole/trimethoprim); EA, essential agreement; FEP, cefepime; IMC, isothermal microcalorimetry; ME, major error; mE, minor error; MER, meropenem; MIN, minocycline; NT, not tested; PTZ, piperacillin/tazobactam; SUL, sulbactam; VME, very major.

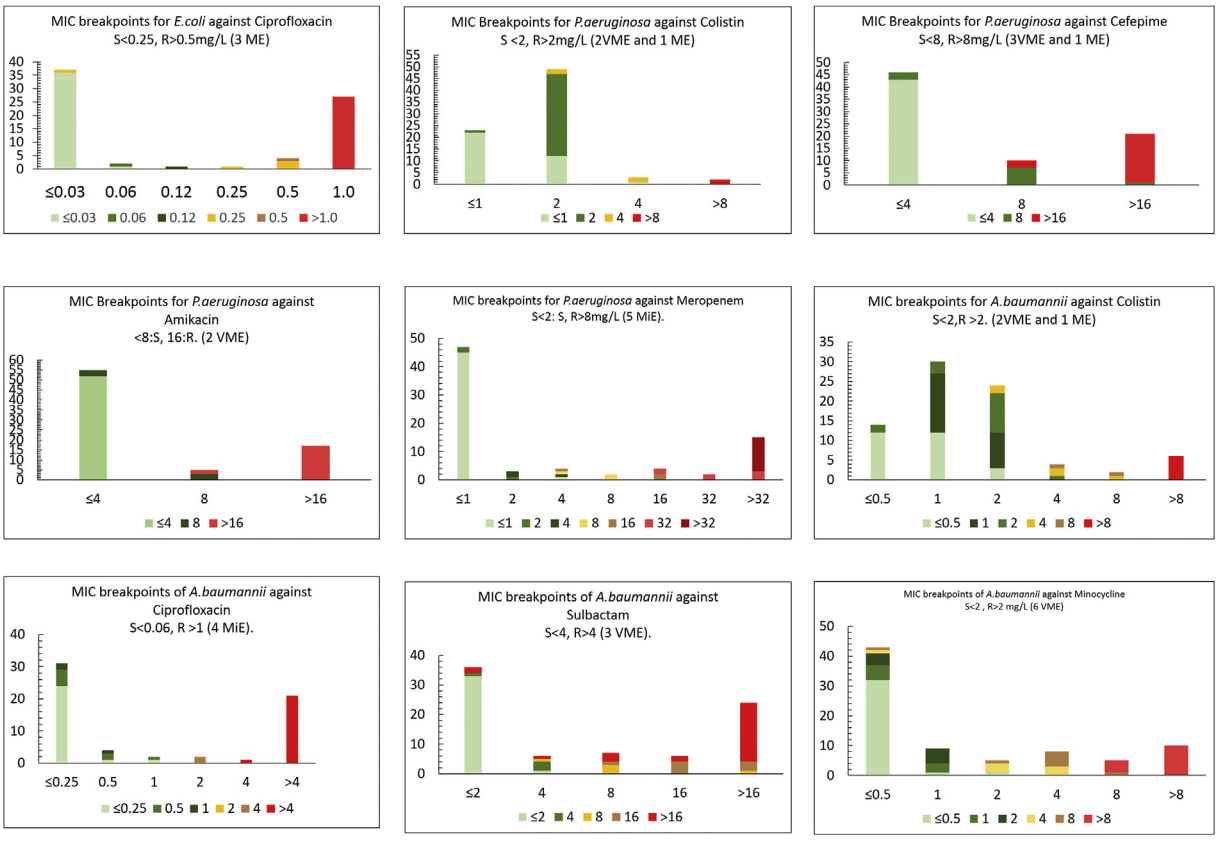


**Fig. 1.** Comparison of IMC and ISO-BMD methods for determining MICs. IMC, isothermal microcalorimetry. ISO-BMD, ISO-broth microdilution.

## Discussion

We present here the accuracy of a multichannel IMC device, calScreener, for determination of MIC for bacterial pathogens against various antibiotics. A multicentre evaluation of the method was performed by including four commonly encountered Gram-negative bacterial species that cause clinical infections and tested against a panel of clinically relevant antibiotics. Average essential agreement and categorical agreement of the IMC method were 96.6% and 97.1% when tested using the clinical isolates. The method demonstrated excellent reproducibility when performed and interpreted independently at each of the four study sites with the same panel of reference strains and antibiotics.

Categorical agreements of the IMC method varied between the antibiotics tested (Table 3), bacterial species (Table 4) and most importantly the time (Fig. 3) at which the results were determined in the present study. In general, the performance of any new AST method can significantly vary or be biased based on the characteristics of challenge isolates included for the evaluation [1]. One of the major strengths of our study is that we used challenge isolates from the frozen collections of four European referral hospitals (to avoid the influence of clonality) that were well distributed (Table 2), with reference to their MICs against important antibiotics of clinical relevance. By doing so, we were able to investigate the categorical agreement of the IMC method using calScreener against a challenging strain collection, thus provoking the system maximally. IMC data from four (1.2%) of 320 isolates did not meet the technical inclusion criteria and hence were not included in the final analysis. A re-run of these four samples could resolve the technical uncertainty and allow for a MIC determination. Overall the error rates of the IMC method by the end of 18 hours ranged between 1% and 3.7% among the four bacterial species tested. The IMC method performed well in predicting the MIC among *Enterobacterales* isolates ($n = 159$), sparing the three major errors when tested against ciprofloxacin and two minor errors against meropenem.

EUCAST has identified some situations when reproducibility of AST, including sometimes also BMD, is particularly challenging. These MIC values or sometimes ranges of values are referred to as the area of technical uncertainty for certain antibiotics that are more prone to generate categorical errors while testing using the BMD method. Four of the 77 *P. aeruginosa* isolates included in the study had an MIC of 4 mg/L, determined using the reference method against colistin, which is recognized as an area of technical uncertainty for colistin among *P. aeruginosa* by EUCAST. The IMC method identified two of these four isolates as susceptible (false) and one isolate as resistant (false), leading to 33.3% VME and 1.4% ME. The concept of the area of technical uncertainty has only been available since January 2019, so it has not yet been fully established whether it should be considered when calculating errors in AST. Nevertheless, some of the observed discrepancies could clearly be explained by the problems of reproducible AST with colistin and *P. aeruginosa,* which apply to all MIC methods.

With increasing number of infections due to multidrug-resistant *A. baumannii,* minocycline and sulbactam have sometimes been suggested as therapeutic options, mostly in combination with another agent. MIC breakpoints for these drugs against *A. baumannii* are currently not available from EUCAST [10,11]. The IMC method generated VME more frequently while determining MICs for sulbactam and minocycline. Establishing susceptibility for these two antibiotics was reported to be problematic using several available AST methods [12]. We foresee the need for further evaluation of the IMC method for its accuracy in predicting MICs for

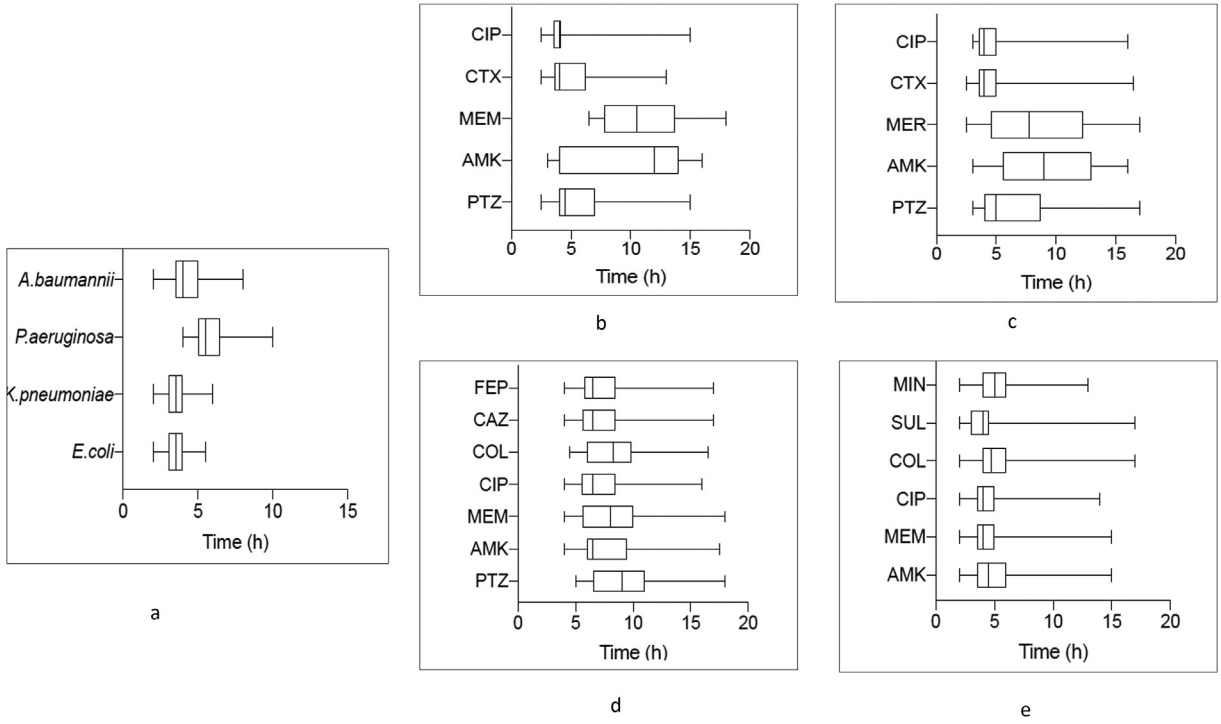


**Fig. 2.** TTD of MICs among four bacterial species tested. (a) TTD of stable metabolic activity among each of four bacterial species tested in absence of antibiotics. (b–e) Representation of range and median time needed for determination of MICs against each antibiotic tested for (b), *Escherichia coli* (c), *Klebsiella pneumoniae* (d), *Pseudomonas aeruginosa* (e), *Acinetobacter baumannii*. TTD, time to detection.

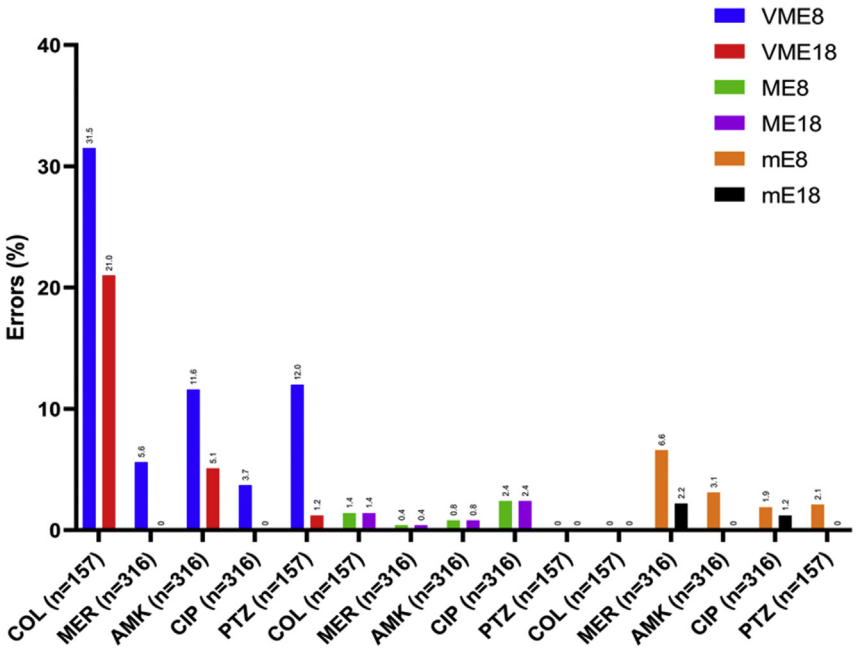


**Fig. 3.** Comparison of categorical errors by isothermal microcalorimetry (IMC) method at 8- and 18-hour time points.

these two antibiotics, using a large comprehensive collection of *A. baumannii* isolates comprising both wild-type and resistant phenotypes to draw any conclusions.

Essential agreement of IMC method with the reference method for the antibiotics tested in the present study ranged between 90% and 100%, with an average of 96.3%. It is possible that the results derived from our study, with special reference to the essential agreement of the IMC method, could have been slightly different if only the exact MICs were determined instead of truncations in the highest and lowest MIC dilutions. We recognize this as one of the limitations of our study. Reproducibility of the IMC method was performed using five reference strains, tested independently at each of the four study sites using the same batch of media (MHB) and working solutions of antibiotics prepared and distributed

across all the study sites. Further, all the tests were performed by skilled researchers with expertise in performing BMD assays. Considering these factors, it is not surprising that both the BMD and IMC methods demonstrated excellent reproducibility in our study. Nevertheless, it is important to remember that the IMC method *per se* is highly sensitive and, like BMD, can produce erroneous results from minute contaminations and/or pipetting errors.

In the present study, we used 18 hours as the endpoint for MIC determination, but attempts were made to measure MICs also after 8 hours. We did not observe a change in the percentage of ME due to prediction of MIC at 8 and 18 hours by the IMC method. However, a considerable decline in the frequency of VME with an increase in time for prediction from 8 to 18 hours was observed while analyzing the results for all the five antibiotics analyzed at two different time points (Fig. 3). From these findings, it is possible to postulate that the time needed for determining MICs may most likely be strain dependent within a given bacterial species. Even though some isolates require 18 hours, many isolates could be correctly classified in less time than the reference method. Given this observation, we foresee the need for further evaluation of the IMC method for its utility in predicting MICs at various time points among Gram-negative bacilli, particularly against the last-resort antibiotics.

In conclusion, we have shown that the determination of MICs based on metabolic activity of bacteria could be achieved using the new IMC device, calScreener. In some strains, the IMC method was faster than the BMD for determining MICs. calScreener had an additional advantage in that the bacterial growth in the presence of antibiotics could be monitored in real time, which is not possible with the conventional BMD testing. With further improvements in the existing methodology used for testing and the data analysis software, the new method has the potential to decrease turnaround time for determining MICs without compromising the quality of the results.

## Transparency declaration

The project was performed in collaboration with Symcel, which developed the calScreener technology. CGG and RC are members of the Steering Committee of EUCAST. Funding was received from the European Union's Horizon 2020 research and innovation programme (grant 729076). All authors report no conflicts of interest relevant to this article.

## References

[1] Matuschek E, Ahman J, Webster C, Kahlmeter G. Antimicrobial susceptibility testing of colistin—evaluation of seven commercial MIC products against standard broth microdilution for *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Acinetobacter* spp. Clin Microbiol Infect 2018;24:865–70.

[2] Haffler ZJ, Kulengowski B, Ribes JA, Burgess DS. Evaluation of the BD Phoenix™ automated system for determining antimicrobial susceptibility against carbapenem-resistant *Enterobacteriaceae* compared to broth microdilution. Int J Antimicrob Agents 2019;54:249–54.

[3] Braissant O, Wirz D, Göpfert B, Daniels AU. Use of isothermal microcalorimetry to monitor microbial activities. FEMS Microbiol Lett 2010;303:1–8.

[4] Butini ME, Abbandonato G, Di Rienzo C, Trampuz A, Di Luca M. Isothermal microcalorimetry detects the presence of persister cells in a *Staphylococcus aureus* biofilm after vancomycin treatment. Front Microbiol 2019;10:332.

[5] von Ah U, Wirz D, Daniels A. Isothermal micro calorimetry—a new method for MIC determinations: results for 12 antibiotics and reference strains of *E. coli* and *S. aureus*. BMC Microbiol 2009;9:106.

[6] Braissant O, Keiser J, Meister I, Bachmann A, Wirz D, Göpfert B, et al. Isothermal microcalorimetry accurately detects bacteria, tumorous microtissues, and parasitic worms in a label-free well-plate assay. Biotechnol J 2015;10:460–8.

[7] Wadsö I, Hallén D, Jansson M, Suurkuusk J, Wenzler T, Braissant O. A well-plate format isothermal multi-channel microcalorimeter for monitoring the activity of living cells and tissues. Thermochim Acta 2017;652:141–9.

[8] ISO 20776-2 Clinical laboratory testing and *in vitro* diagnostic test systems—susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices—part 2: evaluation of performance of antimicrobial susceptibility test devices. 2007. https://www.iso.org/standard/41631.html.

[9] Baldoni D, Hermann H, Frei R, Trampuz A, Steinhuber A. Performance of microcalorimetry for early detection of methicillin resistance in clinical isolates of *Staphylococcus aureus*. J Clin Microbiol 2009;47:774–6.

[10] Tsakris A, Koumaki V, Dokoumetzidis A. Minocycline susceptibility breakpoints for *Acinetobacter baumannii:* do we need to re-evaluate them? J Antimicrob Chemother 2018;74:295–7.

[11] Chen H, Liu Q, Chen Z, Li C. Efficacy of sulbactam for the treatment of *Acinetobacter baumannii* complex infection: a systematic review and meta-analysis. J Infect Chemother 2017;23:278–85.

[12] Fernández-Cuenca F, Tomás M, Caballero-Moyano FJ, Bou G, Pascual Á. Reporting antimicrobial susceptibilities and resistance phenotypes in *Acinetobacter* spp: a nationwide proficiency study. J Antimicrob Chemother 2017;73:692–7.